

**Supplemental Figure 1**. The temperature response of Rubisco carboxylation ( $v_c$ ) and oxygenation ( $v_o$ ) modeled at  $pCO_2$  and  $pO_2$  expected at the site of Rubisco carboxylation in a C<sub>3</sub> species (25 Pa CO<sub>2</sub> and 21 kPa O<sub>2</sub>; Panel A and B), and a C<sub>4</sub> species (400 Pa CO<sub>2</sub> and 35 kPa O<sub>2</sub>; Panel C and D). The solid black lines are the modeled temperature response of *S. viridis* from this report. Dashed and dotted lines are temperature responses from previous reports assuming a  $k_{catCO2}$  of 3.3 mol CO<sub>2</sub> mol<sup>-1</sup> site s<sup>-1</sup>(Badger and Collatz, 1978; Jorden and Ogren, 1984; Bernacchi et al., 2001 and 2002; Walker et al., 2013).



**Supplemental Figure 2.** An example measurement of PEPc activity on the membrane inlet mass spectrometer at 25 °C. Panel A shows the plot of  $HCO_3^-$  concentration during the assay with the blank and enzymatic rate of  $HCO_3^-$  consumption identified in the first assay point and subsequent arrows identify the points just after the blank and before the catalyzed rates. The expanded section in panel A shows one representative  $HCO_3^-$  concentration and the injections of assay buffer (with NaHCO<sub>3</sub> and glucose 6-phosphate) followed by the leaf extract and initiation of the reaction with PEP. The noise between assays is due to cleaning of the cuvette. The data from panel A are plotted as the Michaelis-Menten response of PEP carboxylation ( $v_p$ ) versus [HCO<sub>3</sub><sup>-</sup>] with the maximum rate of PEPc ( $V_{pmax}$ ) and the Michaelis-Menten constant for HCO<sub>3</sub><sup>-</sup> ( $K_P$ ) labeled.



**Supplemental Figure 3**. The temperature response of the uncatalyzed and carbonic anhydrase catalyzed hydration of CO<sub>2</sub> measured with membrane inlet mass spectrometry. The 1<sup>st</sup> order uncatalyzed rate constant for the hydration of  $CO_2$  under the assay conditions ( $k_{h assay}$ ) was calculated in response to temperature (panel A). The enhancement over the uncatalyzed rate of CO<sub>2</sub> hydration with the addition of leaf extract (solid line, panel B) was used to calculate carbonic anhydrase  $CO_2$  hydration activity ( $v_h$ ) in response to temperature (dashed line, panel B). The calculated  $v_h$  took into account changes in pH (solid line) of the assay buffer and [CO<sub>2</sub>] (dashed line) with temperature (panel C). The estimated change in CO<sub>2</sub> molarity used a pKa assuming 0.1 M ionic strength and the temperature dependency as described by Harned and Bonner (1945). The circles represent average values of three replicates with  $\pm$  SE. The p-value refers to the significance of the temperature response from zero and the adjusted  $R^2$  value describes the amount of variation in the measured parameter explained by the model. The lines represent the modeled fits of the measured data using the equation parameter= $k_{25} \exp[(E_a)$  $(T_k-298.15))/((298.15RT_k))]$  for  $k_h$ , enhancement, and  $v_h$ , and y=mx+b for pH. The  $k_{25}$  and  $E_a$ were 0.0428 s<sup>-1</sup> and 91.3 kJ mol<sup>-1</sup> for  $k_h$ , 0.512 and -59.3 kJ mol<sup>-1</sup> for enhancement, and 1004.327 mmol m<sup>-2</sup> s<sup>-1</sup> and 47.2 kJ mol<sup>-1</sup> for  $v_h$ . For pH, *b*=8.49, and *m*=-0.016.